SOME OBSERVATIONS ON THE PLASMODIA AND OTHER BLOOD PARASITES OF SPARROWS.

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Introduction.

Within a few years of the discovery of the parasite of human malaria Danilewsky found similar plasmodia in birds. Since then the study of bird malaria has proceeded fairly steadily, drawing impetus from the belief that the results obtained with the disease in birds would help to solve some of the problems of human malaria. However, little work has been done on bird malaria in Australia. Although protozoa have been found in the blood of many species of local birds, it is only rarely that plasmodia have been described. Gilruth, Sweet and Dodd (1910) found a plasmodium in the musk duck (*Biziura lobata* Shaw) and named it *Plasmodium biziurae*. This species was later found in the black swan (*Chenopsis atrata* Shaw) by Cleland (1915). Besides this, Johnston (1909) had reported finding a plasmodium in a sparrow (*Passer domesticus* Linn.). Though he described it at first as *Plasmodium praecox*, he later (Johnston and Cleland, 1909) thought that it was a new species and called it *Plasmodium passeris*. Cleland in 1915 reported finding the same species, again from a sparrow. Breinl (1913) described and figured *P. praecox* from the grey falcon (*Falco hypoleucus* Gould).

At least thirty-three species of plasmodia have been described from birds, but probably not more than fourteen of these species are valid. Four species that are usually recognized as valid have been found in the sparrow. These are *P. relictum* Grassi and Feletti, *P. cathemerium* Hartman, *P. rouxi* Sergents and Catanei, and *P. elongatum* Huff. *P. relictum* and *P. cathemerium* are common in passerine birds and have been found in many parts of the world. *P. elongatum* has also been reported from a variety of passerine birds, but is less common than the former. *P. rouxi* seems to be restricted to the sparrows of Algeria.

Methods.

In the present investigation the local sparrows were examined for blood protozoa, particularly the plasmodia, and an attempt was made to find invertebrate hosts of any species of the latter.

The birds were trapped alive, and smears of blood were made from the tarso-metatarsal vein, stained with Giemsa, and examined under the microscope for at least ten minutes. Usually the birds were kept for some time and blood smears were then made at intervals of a few days. In a number of cases (39), impression smears were made from some of the internal organs (liver, spleen and either bone marrow or brain) and examined as usual.

Work on the invertebrate host was confined entirely to laboratory experiments, the object of which was to determine whether certain of the local mosquitoes were susceptible to infection. Larvae or pupae of some of the local species (*Culex fatigans* Wiedmann, *Aëdes* (*Finlaya*) *notoscriptus* Skuse, *Aëdes* (*Stegomyia*) *aegypti* Linn., *Aëdes* (*Pseudoskusea*) *concolor* Taylor and *Anopheles annulipes* Walker) were collected in the field, larvae were reared to pupae and the adults were allowed to emerge into a mosquito cage. They were left at room temperature but the humidity was kept as high as possible. Water, and food in the form of raisins, were provided. However, before the mosquitoes were

given the opportunity to feed on an infected bird (either a canary or a sparrow), they were usually deprived of food and water for 24 hours. Two feeding methods were used. In the first, the bird was kept in the dark, confined in a small cage, in order to restrict its movements as much as possible; and there exposed to the mosquitoes overnight. The second method was that described by Huff (1927). The bird was immobilized by wrapping it in gauze and the breast region was bared by wetting and parting the feathers. The bird was then tied on top of the mosquito cage in such a way that the mosquitoes could reach the exposed breast, and left in this position for an hour. The feedings were made either in daylight or in the late afternoon in complete or semi-darkness. Any mosquitoes that engorged were isolated and kept in cages at room temperature. After 5 to 20 days they were dissected and both the mid-gut and salivary glands examined to determine whether they had become infected.

Results.

In addition to sparrows a few other birds were trapped. The results of the examination of all these birds are given in Table 1. *P. relictum* and *P. cathemerium*

Table 1.
Incidence of Infection in Birds.

Species.	Number Examined.	*P. cathemerium-relictum.*	*Plasmodium* sp.	*Haemoproteus.*	"Toxoplasma."
Sparrow (*Passer domesticus* Linn.)..	91	60	5	—	27
Blue Wren (*Malurus cyaneus* Latham)	3	—	—	—	—
White Eye (*Zosterops lateralis* Latham)	3	—	—	1	—
Starling (*Sturnus vulgaris* Linn.) ..	2	1	—	—	—
Willie Wagtail (*Rhipidura leucophrys* Latham)	1	—	—	—	—
Total	100	61	5	1	27

have not been recorded separately, both species being included under *P. cathemerium-relictum* for the following reason. *P. cathemerium* was separated from *P. relictum* by Hartman (1927), who declared that the shape and arrangement of the pigment of the two species were different. This was the only morphological distinction that he made. The differences may be tabulated as follows:

	P. cathemerium.	*P. relictum.*
Gametocytes.	Rod-shaped pigment granules which are longer and more pointed at the ends in micro- than in macro-gametocytes.	Nearly spherical pigment granules which are usually grouped close together.
Trophozoites.	Pigment appears as an amorphous mass, or at most not more than a few masses which are close together.	Pigment is scattered and frequently in small granules.

P. cathemerium is still regarded as a valid species (Bishop, 1942), but the sole morphological distinction now stressed is the difference in pigment shape in the gametocytes, particularly the microgametocytes. There is one fairly definite biological difference. Strains of *P. cathemerium* isolated from wild birds have always been synchronous, i.e., the parasites tend to keep in step, all being at the same stage of development at the same time; but the *P. relictum* strains usually show little, if any, synchronism. Occasionally synchronous strains of *P. relictum* have been isolated, but these differ from *P. cathemerium*, which liberates its merozoites in the evening, by liberating them in the morning. In a few cases smears were made at intervals throughout the day to see whether the strains were synchronous and to find the time of merozoite liberation. Two synchronous strains were found, both of the cathemerium type, i.e., liberating their merozoites in the evening. These strains were studied both in the birds

originally infected and also in canaries or sparrows inoculated from them. Similarly two asynchronous strains were carefully studied. The pigment in the gametocytes of both the synchronous and the asynchronous strains was very variable in size and in shape, at times varying from fine to coarse, and from round to somewhat elongate in the same cell. However, there seemed to be a tendency for the pigment in the gametocytes of the cathemerium strains to be more elongate than that in the relictum strains, though it was necessary to study a number of gametocytes to draw any conclusion. In fact, the two species in Sydney sparrows do not show the clear-cut morphological difference described in the literature of other countries. Since most of the birds were not given such a thorough examination, it was thought it would be less misleading if *P. relictum* and *P. cathemerium* were not separated in the results.

In some cases when the parasites were few, it was not possible to make a diagnosis beyond *Plasmodium* sp. though there is no reason to believe that these were neither *P. relictum* nor *P. cathemerium*.

Table 2 gives the results of the experimental work on the invertebrate host of *P. cathemerium-relictum*. The sixth and seventh columns give the results of the dissections on the fed mosquitoes. The result is recorded as positive if oöcytes could be seen on the mid-gut or sporozoites in the salivary glands. In most cases an asynchronous strain (*P. relictum*) was the source of the gametocytes, but similar results were obtained with a synchronous strain (*P. cathemerium*).

TABLE 2.
Results of Feeding Experiments with Mosquitoes.

Species.	No. that Fed.	No. that failed to Feed.	Percentage Feds.	No. of Feds Dissected.	Number Positive.	Number Negative.
Culex fatigans ..	150	21	88	107	75	32
Aëdes notoscriptus ..	27	117	19	27	0	27
Aëdes concolor ..	1	27	4	1	1	0
Aëdes aegypti ..	5	1	83	5	0	5
Anopheles annulipes	0	111	0	—	—	—

Discussion.

A very high percentage (71%) of sparrows were infected with plasmodia; much higher than appears to have been usually found elsewhere. For instance, Manwell and Herman (1935), working at Syracuse, N.Y., found only 6 infected (all with *P. relictum*), out of 244 examined. They state that non-migratory birds like sparrows are not as commonly infected as migratory birds.

Examination of smears made from the birds at intervals of a few days often revealed infections that would otherwise have been missed. Presumably these extra positives were due to the infection being in the latent stage at first and later relapsing or, less often, to the bird being caught while still incubating the disease. On the other hand, the examination of smears from the internal organs did not reveal any infections with plasmodia that had been missed in the smears made from the tarso-metatarsal vein. These results are at variance with those of Hewitt (1940), who found more positive plasmodial infections by examining smears from the internal organs as well as from the peripheral blood.

Besides plasmodia the only other blood parasites seen in the sparrows were oval organisms that were usually found in the cytoplasm of mononuclear leucocytes, the nuclei of which they indented. Sometimes they appeared to be lying free in the blood. They were seen both in the presence of, and in the absence of, associated malaria parasites. Organisms similar to this have been frequently described. They resemble the type II avian "Toxoplasma" of Wolfson (1940) and the forms of "Toxoplasma" described and photographed by Manwell (1939). They were usually more numerous in the internal organs than in the blood from the tarso-metatarsal vein. In some cases they could be found only in the internal organs, but on the other hand, though more rarely, they might be found only in the peripheral blood.

A starling (*Sturnus vulgaris* Linn.) was found infected with *P. relictum*, which was successfully transmitted to sparrows by blood inoculation. Manwell (1934) and Manwell and Herman (1935) have reported both *P. relictum* and *P. cathemerium* from the starling in the United States of America, though they found that infections were rare.

One haemoproteus infection was found in a white eye (*Zosterops lateralis* Latham). Forms ranging from small round young forms to mature gametocytes were seen in the blood. The mature gametocytes lay beside the nucleus of the parasitized erythrocyte and encircled its ends. The pigment was coarse and tended to be rod-shaped. No schizonts were seen in smears of the liver, spleen or brain. Partial confirmation of the fact that it was not a plasmodium was obtained by inoculating blood from the white eye into a canary, which failed to develop any infection. This haemoproteus is probably identical with that described from the same species by Cleland and Johnston (1910).

It will be convenient at this point to discuss the present position of *Plasmodium passeris* described by Johnston and Cleland from a sparrow. It was originally described as *P. praecox* (Johnston, 1909). The name *P. praecox* was given by Grassi and Feletti both to a plasmodium of sparrows and to the plasmodium causing malignant tertian fever in man. These two species are quite distinct. Johnston and Cleland were unaware of this confusion in nomenclature and, since the only description of *P. praecox* available to them referred to the parasite of malignant tertian fever, they concluded that their species was new. However, this parasite of sparrows had been already described under the names of *P. praecox* and also *P. relictum* by Grassi and Feletti (1890–1891). The name *praecox* has now been generally dropped, partly owing to the confusion it has caused. The malignant tertian parasite is now called *P. falciparum* Welch, while the bird parasite is now usually known as *P. relictum* Grassi and Feletti. Since then, as has been mentioned already, a second species, *P. cathemerium*, which is closely related to *P. relictum*, has been described; the morphological distinction between them resting on differences in the pigment. The pigment of *P. passeris* was described as consisting of small granules, a description that could fit either species. *P. passeris* becomes a synonym for *P. relictum* or *P. cathemerium*.

Of the mosquitoes tested, *Culex fatigans* was by far the best vector under laboratory conditions. It would bite readily and a large proportion of the mosquitoes that fed became infected. It was hard to get the other successful vector (*Aëdes concolor*) to bite. Possibly this was due to the laboratory conditions: the mosquitoes suffered a heavy mortality during the period without food and water. As they are salt-water breeders they are probably, at best, of secondary importance as a vector of the malaria parasite of sparrows in nature. *Aëdes aegypti* seemed to bite birds quite readily but of the few tested none became infected. Huff (1927) has shown that they are susceptible to overseas strains of *P. relictum* and *P. cathemerium* though they are poor vectors. He found that only 6% became infected. None of the *Aëdes notoscriptus* became infected and it was hard to induce them to bite. On one occasion, using Huff's method, six *Aëdes notoscriptus* failed to feed on a bird within an hour. Immediately afterwards they were given the opportunity of feeding on man under exactly the same conditions. Four of the six fed within half an hour. Possibly they do not normally bite birds.

None of the anophelines would feed on the birds although sixteen different attempts were made under varying conditions as follows. The mosquitoes were kept without food and water for periods up to 48 hours and were, at times, cooled to 4° C. or warmed to 37° C. just before the attempted feeding; the feeding cage was kept either at room temperature, which ranged from 13°–20° C., or put in the incubator at 23°–25° C.; sometimes the humidity was increased by placing a wet towel over the cage. On one occasion eight anophelines that had failed to feed on a bird overnight were given the opportunity to feed on man. Two fed within one hour. Although these experiments took place under highly artificial conditions, they suggest the possibility that *Anopheles annulipes* does not normally bite birds.

In this work very few birds other than sparrows have been examined, but earlier workers, in particular Johnston and Cleland, have examined many species of native birds

from the vicinity of Sydney for plasmodia without finding any infected. So, although the local sparrows in Sydney are heavily infected, their infection does not seem to have been transmitted to the indigenous species of birds. It may be remarked in passing that it proved impossible to infect a zebra finch (*Taeniopygia castanotis* Gould) by inoculation of blood from a sparrow infected with *P. relictum*.

Summary.

(1). Of 100 birds examined for blood protozoa 66 were positive for *Plasmodium*, 27 for "Toxoplasma" and 1 for *Haemoproteus*.

(2). *Culex fatigans* is an efficient laboratory vector of *P. relictum* and *P. cathemerium*.

(3). It is impossible to distinguish clearly between *P. relictum* and *P. cathemerium* in Sydney sparrows by the usual morphological character applied overseas.

References.

Bishop, A., 1942.—Chemotherapy and Avian Malaria. *Parasitology*, 34 : 1.

Breinl, A., 1913.—Parasitic Protozoa encountered in the Blood of Australian Native Animals. *Aust. Inst. Trop. Med. Rep.* for 1911 : 34.

Cleland, J., 1915.—The Haematozoa of Australian Birds. No. 3. *Trans. Roy. Soc. S. Aust.*, 39 : 25.

————, and Johnston, T. H., 1910.—The Haematozoa of Australian Birds. No. 1. Ibid., 34 : 100.

Gilruth, J., Sweet, G., and Dodd, S., 1910.—Notes on Blood Parasites. *Proc. Roy. Soc. Vict.*, 23 : 231.

Grassi, B., and Feletti, K., 1890-1891.—*Bulletin mensuel de l'Académie Gioena des Sciences Naturelles de Catane.* (Quoted by Ed. and Et. Sergent and Catanei, 1929, *Archiv. Inst. Past. d'Algérie*, 7 : 223.)

Hartman, E., 1927.—Three Species of Bird Malaria, *P. praecox*, *P. cathemerium*, n. sp., and *P. inconstans*, n. sp. *Arch. Protistenk.*, 60 : 1.

Hewitt, R., 1940.—Studies on Blood Protozoa from Mexican Wild Birds. *J. Parasit.*, 26 : 287.

Huff, C., 1927.—Studies on the Infectivity of Plasmodia of Birds for Mosquitoes, etc. *Amer. J. Hyg.*, 7 : 706.

Johnston, T. H., 1909.—*Rec. Aust. Mus.*, 7 : 344.

————, and Cleland, J., 1909.—Notes on Some Parasitic Protozoa. Proc. Linn. Soc. N.S.W., 34 : 501.

Manwell, R., 1934.—How Many Species of Bird Malaria are there? *J. Parasit.*, 20 : 344.

————, 1939.—Toxoplasma or Exoerythrocytic Schizogony in Malaria? *Riv. Malariol.*, 18 : 76.

————, and Herman, C., 1935.—Occurrence of Avian Malarias in Nature. *Amer. J. Trop. Med.*, 15 : 661.

Wolfson, F., 1940.—Organisms described as Avian Toxoplasma. *Amer. J. Hyg.*, 32 : 88.